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Screening of Exopolysaccharide-Producing *Lactobacillus* and *Bifidobacterium* Strains Isolated from the Human Intestinal Microbiota[▽]

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Using phenotypic approaches, we have detected that 17% of human intestinal *Lactobacillus* and *Bifidobacterium* strains could be exopolysaccharide (EPS) producers. However, PCR techniques showed that only 7% harbored genes related to the synthesis of heteropolysaccharides. This is the first work to screen the human intestinal ecosystem for the detection of EPS-producing strains.

The human intestinal microbiota has around 10 times more cells than the human body (2), and its genome ("microbiome") harbors at least 100 times more genes than our own genome (5). However, a significant fraction of intestinal bacteria has not been described yet, making it difficult to understand the mechanisms of communication among the microbiota, host cells, and intestinal environment (21). *Bifidobacterium* and *Lactobacillus* species are common inhabitants of the gastrointestinal tract, and they have received special attention because of their long history of safe use in foods and probiotic effect (11). Some probiotic strains are able to adhere to intestinal mucus, and we have postulated that exocellular polysaccharides isolated from lactic acid bacteria (LAB) and bifidobacteria interfere with the adhesion of probiotics and pathogens to human intestinal mucus (9, 10). Therefore, the production of exopolysaccharides (EPS) could also be an interesting property to consider for the selection of putative probiotic strains (17). Currently, the most suitable approach to the search for novel EPS-producing (EPS+) strains is the exploration of wild bacteria (6). The aim of this study was to investigate the EPS+ capabilities among *Lactobacillus* and *Bifidobacterium* strains isolated from the human intestinal ecosystem as the initial step for further investigation of the roles of EPS in bacterium-host interactions and in human health.

We have employed 362 *Lactobacillus* and *Bifidobacterium* strains, previously isolated from fecal and mucosal samples of healthy adult volunteers (3, 4), and 4 reference EPS+ bifidobacteria (*Bifidobacterium animalis* subsp. *lactis* IPLA-R1, *Bifidobacterium longum* NB667 [10], *B. longum* BL1, and *B. longum* 667Co) for screening of EPS production in solid media. Strains were grown at 37°C for 72 h under anaerobic conditions on the surface of MRS agar containing 0.25% L-cysteine (MRSC agar) and supplemented with 2% glucose, fructose, lactose, or sucrose added separately. Sixty putative EPS+ strains were detected, most of them being mucoid (92%) and only 5 (8%) having a "ropy" character. The percentage of putative EPS+ strains (17%) was similar to that reported in the literature for EPS+ LAB isolated from food environments (1, 6, 12) and from animal origins (14). Amplification, sequencing, and comparison with database sequences for the V1-V2 variable region of the 16S rRNA gene (7, 19) identified 35 out of our 60 putative EPS+ strains as belonging to the genus *Bifidobacterium* and 25 as belonging to *Lactobacillus* (with nucleotide identity at the species level higher than 98%). The EPS+ bifidobacteria were *Bifidobacterium pseudocatenulatum* (51%), *B. longum* (40%), *B. animalis* (6%), and *Bifidobacterium adolescentis* (3%). These results were in general coincident with those for the most abundant species found in the fecal samples of the donors (4). The EPS+ lactobacilli were *Lactobacillus casei* (48%), *Lactobacillus rhamnosus* (24%), *Lactobacillus plantarum* (16%), *Lactobacillus gasseri* (4%), *Lactobacillus acidipiscis* (4%), and *Lactobacillus vaginalis* (4%). Contrary to what was found for bifidobacteria, *L. gasseri* was the most abundant lactobacilli in the intestinal population of donors but one of the lowest EPS producer species. Remarkably, all *L. plantarum* human isolates were putative EPS+ strains.

A screening for glycansucrase and glycosyltransferase genes related to the synthesis of homo- and heteropolysaccharides, respectively, was performed on the 60 putative EPS+ strains of human origin and on the 4 reference bifidobacteria. PCR techniques were employed to detect fragments of genes coding for glucansucrases (14) and fructansucrases (13) as well as for the glycosyltransferase involved in the synthesis of β-D-glucan (20), but the results obtained were not positive. The pGT primers were employed to amplify the genes coding for the "priming glycosyltransferase," which catalyzes the transfer of a sugar-1-phosphate to a lipophilic carrier molecule anchored in the cellular membrane, this being the first step for the assembly of the repeating unit that builds many heteropolysaccharides (8). Amplifications were obtained for 25 human isolates (11 bifidobacteria and 14 lactobacilli) and the 3 reference strains of *B. longum.* The predicted amino acid sequences of the PCR

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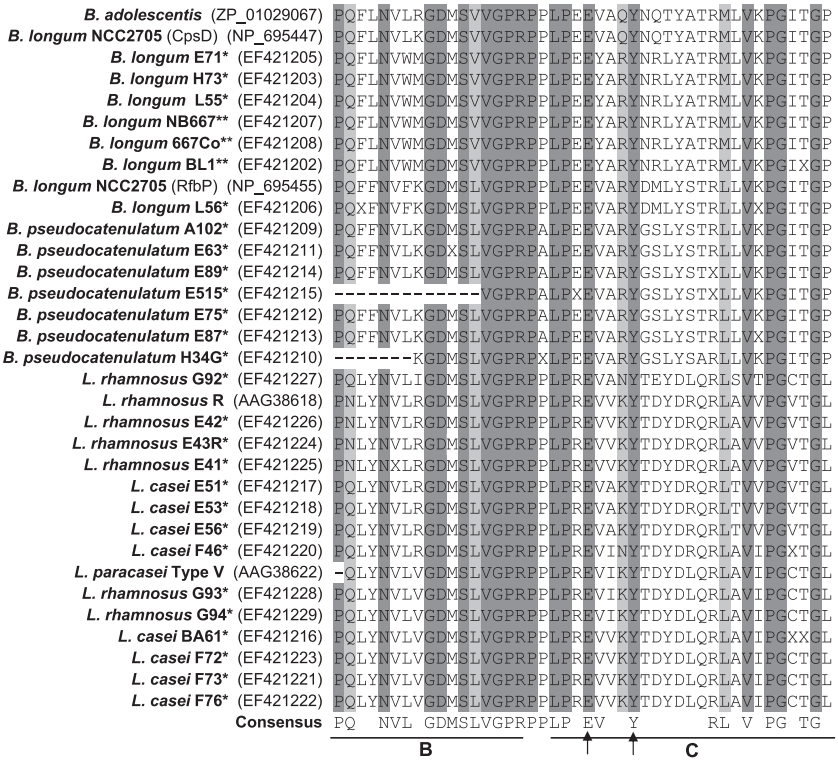

FIG. 1. Alignment of an internal fragment close to the C-terminal amino acid sequences (44 amino acids each) of priming glycosyltransferases deduced from PCR amplifications with pGT primers for 28 strains employed in this study: 25 of human origin (marked with one asterisk) and 3 from the reference strains *Bifidobacterium longum* NB667 (NIZO food research culture collection), *B. longum* 667Co, and *B. longum* BL1 (IPLA culture collection) (marked with two asterisks). The internal fragments close to the C-terminal amino acid sequences for some LAB and bifidobacterial strains held in databases are also included in the alignment. The GenBank accession numbers of all strains are shown in parentheses. The shading identifies amino acids that are identical (dark gray) or conserved (light gray). The letter X indicates an undetermined amino acid. Arrows show the amino acid residues glutamate (E) and tyrosine (Y).

products presented high identity with the partial C-terminal regions of the glycosyltransferases (Fig. 1). Those of the bifidobacteria showed identity (83% to 95%) with two sequences from *B. longum* NCC2705, those of the undecaprenyl-phosphate sugar phosphotransferase RfbP and the galactosyltransferase CpsD. Most sequences from the *Lactobacillus* strains showed identity (93% to 100%) with that of the putative undecaprenyl-phosphate glycosyl-1-phosphate transferase from *L. rhamnosus* or with that of the priming glycosyltransferase of *Lactobacillus paracasei*. Within all these sequences, parts of blocks B and C involved in the interaction with the lipid carrier and in the recognition of the sugar specificity, respectively, were found (8, 18). A glutamate residue present in block C (8) has been proposed as one of the candidates for the catalytic residues in the priming glycosyltransferase of *Lactococcus lactis* (16). Finally, the 28 sequences of our EPS+ strains showed a tyrosine in block C which is conserved in the sequences of priming galactosyltransferases but not in those of glucosyltransferases (16). All these data suggest that our EPS+ strains can carry genes involved in the synthesis of heteropolysaccharides. A phylogenetic tree was constructed with these amino acid sequences (Fig. 2), including some sequences from LAB and bifidobacteria reported in databases. The glycosyltransferase sequences of lactobacilli and bifidobacteria clustered separately. All strains of *B. pseudocatenulatum* and the strain *B. longum* L56 were closely related to the RfbP sequence of *B. longum* NCC2705. The remaining *B. longum* strains, including the reference strains, were more related to the CpsD sequence from *B. longum* NCC2705. The sequences of our *L. casei* and *L. rhamnosus* strains were grouped in several closely related clusters and appeared more differentiated from other species of this genus. *L. rhamnosus* strains E41, E42, and E43R isolated from the same individual were similar to group 2 of the classification by the sequence homology of Provencher and coworkers (8), but none of our isolates fit in their group 1 category. The *L. rhamnosus* strains G93 and G94 were closely related to the sequence of *L. paracasei* type V and also with some intestinal strains of *L. casei*. However, *L. rhamnosus* G92, isolated from the same individual as G93 and G94, appeared clearly differentiated from the other intestinal *L. rhamnosus* and *L. casei* strains. Our results showed variability in the sequences of glycosyltransferases among strains isolated from different individuals and differences among strains from the same individual. Divergences of priming glycosyltransferase sequences among closely related microorganisms have been previously reported by other authors, and more than one potential priming glycosyltransferase can be present in a unique strain (8).

Finally, 2 reference strains and 21 putative EPS+ isolates, originating from different individuals and selected on the basis of their differential carbohydrate fermentation patterns (data not shown), were employed in order to demonstrate their abil-

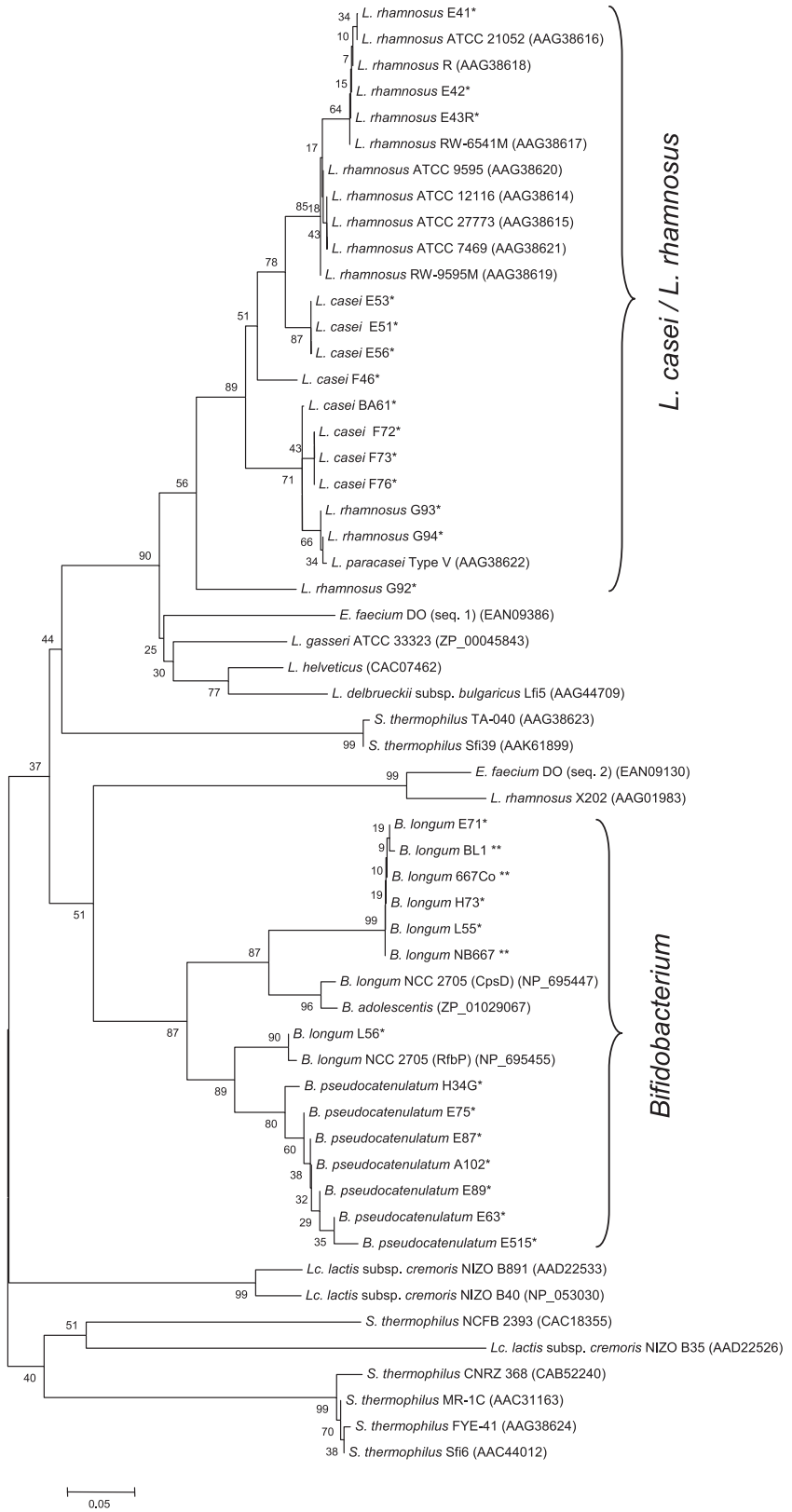

FIG. 2. Phylogenetic tree of an internal fragment close to the C-terminal amino acid sequences (44 amino acids each) of priming glycosyltransferases obtained by PCR amplifications with pGT primers for 28 strains employed in this study: 25 from human origins (marked with one asterisk) and 3 from the reference strains *Bifidobacterium longum* NB667 (NIZO food research culture collection), *B. longum* 667Co, and *B. longum* BL1 (IPLA culture collection) (marked with two asterisks). The GenBank accession numbers of the strains from this study are included in Fig. 1. Sequences of genes from 28 LAB and bifidobacteria held in databases were also included in the phylogenetic analysis, and their GenBank accession numbers are shown in parentheses. The numbers associated with the branches indicate the bootstrap values (confidence limits) resulting from 500 replicate resamplings. The bar scale refers to the number of amino acid substitutions per site.

TABLE 1. Amplification of priming glycosyltransferase genes by PCR and isolation of the EPS fractions produced by selected bifidobacteria and lactobacilli

Strain[a]	Result for amplification with pGT primers[b]	Value for EPS fraction	
		Amt (mg) of powder	% Protein
B. animalis C64MR*	−	77.3	1.9
B. animalis E43*	−	52.9	2.8
B. animalis IPLA-R1	−	47.1	2.2
B. longum/*B. infantis* E44*	−	143.1	6.3
B. longum/*B. infantis* H67*	−	150.4	5.0
B. longum/*B. infantis* H73*	+	217.4	5.7
B. longum/*B. infantis* L55*	+	154.7	3.0
B. longum NB667	+	198.9	2.7
B. pseudocatenulatum A102*	+	71.1	4.3
B. pseudocatenulatum C52*	−	56.6	8.9
B. pseudocatenulatum E63*	+	127.6	2.5
B. pseudocatenulatum E515*	+	65.1	4.0
B. pseudocatenulatum H34G*	+	92.9	5.0
L. casei B61*	+	49.9	8.7
L. casei F72*	+	38.2	6.6
L. casei E51*	+	153.0	6.2
L. rhamnosus E43R*	+	58.4	6.5
L. rhamnosus E41*	+	52.9	6.2
L. plantarum C64MR*	−	106.7	6.5
L. plantarum E112*	−	44.1	2.6
L. plantarum G62*	−	170.1	3.2
L. plantarum H2*	−	77.7	5.3
L. vaginalis C32*	−	38.9	16.0

[a] Strains isolated from the same individual are coded with the same letter followed by different numbers depending on the strain. Strains of human origin are indicated by asterisks. Culture collections: IPLA, Instituto de Productos Lácteos de Asturias (Asturias, Spain); NB, NIZO food research culture collection (Ede, The Netherlands).

[b] A positive result indicates that the sequence of the amplified product corresponds with that of the glycosyltransferase gene. pGT primers were designed by Provencher and coworkers (8).

ities to synthesize EPS (Table 1). EPS fractions were isolated from the surfaces of cultures on MRSC agar (10) to avoid the coisolation of glucomannans present in the medium (15). No correlation between the production of EPS and amplification of the pGT primers was obtained for any *L. plantarum* or *B. animalis* strain and for half of the *B. longum* strains. However, there was a positive correlation between both parameters for the *L. rhamnosus* and *L. casei* groups. Since the pGT primers were designed based on the codon usage of *L. rhamnosus* (8), this result was expected.

In short, the gastrointestinal tract can be a good environment for the isolation of novel heteropolysaccharide-producing *Lactobacillus* and *Bifidobacterium* strains. The degenerated pGT primers (8) could be used to detect some of these strains. However, not all intestinal EPS+ strains can be evidenced with these primers, probably due to sequence heterogeneity of priming glycosyltransferases from different microorganisms.

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